

COLONYZE: AUTOMATED QUANTITATIVE CELLULAR AND COLONY ANALYSIS SYSTEM

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Introduction *In vitro* assays focusing on the prevalence and biological performance of therapeutic stem and progenitor cells have significant clinical and scientific relevance. Quantitative *in vivo* functional assays exist only for hematopoietic cells using irradiated mice. Thus, *in vitro* assays based on counting colony forming units (CFUs) are the current standard for all other systems. CFU assays are severely limited due to their subjective nature, the lack of reproducible standards and their inability to capture biologically relevant information about the heterogeneity that exists between and within stem cell and progenitor populations. They also lack the ability to accurately characterize phenotypic traits of colonies that provide insight into the intrinsic biological potential and performance of their respective colony-founding stem or progenitor cells. Our aim is to develop a customizable, quantitative, automated imaging software for cell and CFU-based assays, which includes detection and analysis of critical cell signaling markers (kinases, phosphatases, transcription factors, etc., via GFP transfection or fluorescent dyes and antibodies) with colony- and cell-level editing capabilities.

Methods LabTek™ culture chambers are imaged using a high resolution CCD digital camera on a motorized microscope equipped with various fluorescent filters. Image tiles are background corrected and montaged/stitched into a single chamber image. *Colonyze*™ software uses a segmentation algorithm to identify colonies, nuclei, cytoplasm and other cellular components. Non-cell artefacts (lint, debris, optical aberrations) are identified and removed. Images are then manually reviewed in *Colonyze*™, whereby object segmentation is confirmed and adjusted accordingly (addition or deletion of cells, colonies, etc.). Data is exported as a text file, then imported, analyzed and graphed in excel.

Results *Colonyze*™ has been used successfully to study human adult Connective Tissue Progenitor (CTP) cells and the effects of Wnt signaling, hypoxia, magnetic cell-selection, and CTP homing to a defect site in a parabiotic mouse model. Customizable metrics include, but are not limited to: colony forming efficiency, cells per colony (proliferation), cell density (migration), number of non-colony cells, nuclei size/area and % area of given cellular differentiation markers (Alkaline Phosphatase, GFP-tagged proteins, etc.).

Discussion Basic academic and industrial research studies aiming to accurately and quantitatively assess changes in the biological performance of cells will benefit from using *Colonyze*™. By identifying individual cells and colonies over a wide range of morphologies and distinguishing between free cells and those within colonies, *Colonyze*™ offers a more accurate and reliable means of providing quantitative cell-based analyses. Additionally, by accurately quantifying cell signaling and differentiation markers at the cell and colony levels, *Colonyze*™ could enhance current cell-line and anti-body production quality control and assurance protocols. Future work includes analysis of additional cellular markers (β -catenin, Sox-9, etc.), application to live-cell, large field-of-view sequences and recruitment of additional off-site β -testers with innovative applications of the Software.

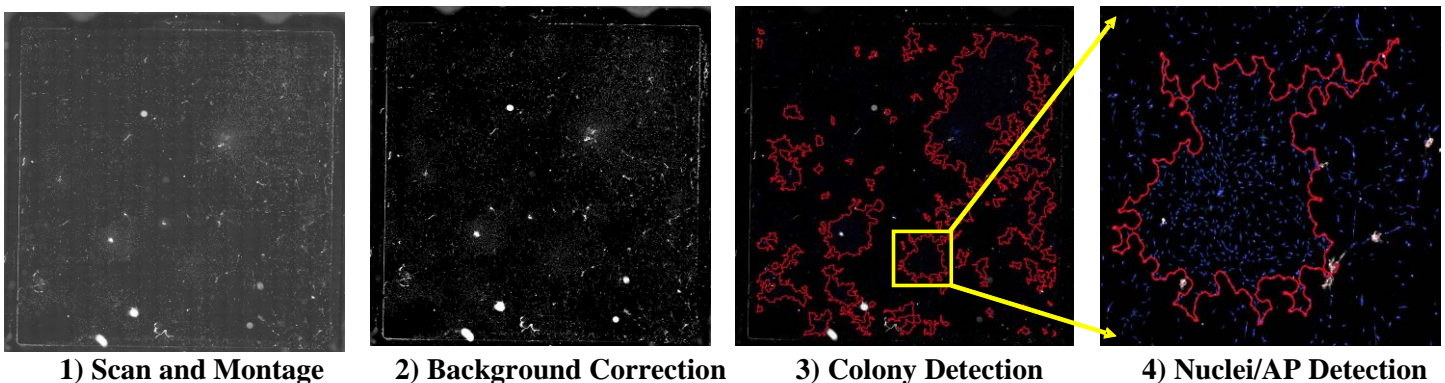


Fig. 1: Image procession outlining cell and colony detection/analysis using the Colonyze™ software program.